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Bezeichnung: Thiazolidinedione-containing compositions and use thereof

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Die angehefteten Stücke sind eine richtige und genaue Wiedergabe der ursprünglichen Unterlagen dieser Patentanmeldung.

München, den 22. September 2003
Deutsches Patent- und Markenamt
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Im Auftrag

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Zusammenfassung

Die vorliegende Erfindung betrifft Zusammensetzungen, die ein Thiazolidindion und einen Liganden für den RAR/RXR-Rezeptor enthalten, wobei der Ligand in der Lage ist, die Expression des NIS-Gens zu induzieren, und die Verwendung dieser Zusammensetzungen als Diagostikum oder Medikament.

Die Erfindung betrifft ferner ein in vitro-Verfahren zur Diagnose von Karzinomen.

Thiazolidinedione-containing compositions and use thereof

The present invention relates to thiazolidinedione-containing compositions and preparations suitable as diagnostics and medicaments for diagnosis and treatment of primary carcinomas and metastases of glandular carcinomas, in particular carcinomas of the salivary gland, the thyroid gland and of mammary carcinomas. The present invention further relates to an in vitro method for diagnosis of the above carcinomas and a diagnostic system for use in this method.

The present invention is illustrated in more detail by the following description and by the attached claims.

Summary

Sodium/iodide symporter (NIS) molecule is responsible for active iodine uptake in thyroid gland. NIS expression has also been demonstrated in other tissues including salivary glands, kidney, stomach, lactating mammary gland and also in human breast carcinoma. The factors modulating NIS expression and iodine uptake in breast carcinoma cells has not been investigated in detail. Retinoic acid (RA) has been shown to increase the iodine uptake in mammary carcinoma cell line MCF-7. Peroxisome proliferator activated receptor-gamma (PPAR-γ) are known to initiate the transcription of target genes by heterodimerizing with retinoic acid receptors (RAR)/retinoic X receptors (RXR). We investigated the effect of ciglitazone (CIG), a ligand for PPAR-γ on iodine uptake by MCF-7 cells with or without trans-RA (tRA). Treatment of MCF-7 cells with tRA increases iodide uptake about 6 fold. However tRA in combination with CIG increases radio iodide uptake by about 20 fold. CIG alone does not induce iodine uptake in MCF-7 cells. These results indicate that NIS gene

expression is synergistically increased upon treatment of CIG in combination with retinoic acid in breast tumor MCF-7 cells. Moreover, it provides a basis for investigating other compounds capable of inducing NIS gene expression and thereby facilitating the diagnosis and radioiodine treatment of mammary carcinoma.

Introduction

Iodine is actively transported in follicular cells of the thyroid gland by sodium/iodide symporter (NIS) for the biosynthesis of the thyroid hormone (Carrasco N, 1993). Expression of NIS has been utilized over decades for radioiodide-based diagnosis and treatment of differentiated thyroid carcinoma (Mazzaferri EL, 1996). NIS is also functionally expressed in many extrathyroidal tissues like kidney, placenta, salivary gland, gastric mucosa and lactating mammary gland (De La Vieja A, 2000). NIS has been detected in 80% human breast tumors by immunohistochemistry (Tazebay UH and Wapnir IL et al, 2000) but with no known *in vivo* biological function. Breast cancer is the third most frequently occurring cancer in the world and the most common forms of malignancy in females (Perkin et al, 1999). Therefore, expression of functional NIS will be useful in radioiodide diagnosis and treatment of the responsive tumors of breast (recently reviewed by De La Vieja A, et al, 2000; Riedel C, et al, 2001; Heufelder HE, et al, 2001). Recently, induction of NIS gene by *trans*-retinoic acid (tRA) has been reported in MCF-7 cells. tRA induced NIS expression has been suggested to be mediated by two families of nuclear receptors, retinoic acid receptors (RARs) and retinoid X receptors (RXRs) (Kogai T et al, 2000). MCF-7 cells are known to express functional RAR/RXR and PPARγ receptors (Titcomb MW et al, 1994; Kilgore MW et al, 1997). PPARγ by heterodimerizing with RAR/RXR (RXRα, RXRβ, or RXRγ) in presence of appropriate

ligand regulates transcription of target genes (Sato M et al, 2001). PPARγ and RAR/RXR are co-expressed in several epithelial malignancies like brain, breast, prostate and lung carcinoma (Nwankwo JO, Robbins ME, 2001; Inoue K et al, 2001).

The most potent activators of PPARγ are synthethetic thiazolidinedione (TZDs) class of compounds (Murphy GJ and Holder JC, 2000). Ciglitazone (CIG), the first compound of TZDs class was described as a selective inducer of PPARγ (Sugiyama Y et al , 20000). Here we studied whether CIG induces iodine uptake by inducing NIS gene expression with or without tRA treatment in mammary carcinoma cell line MCF-7. These cells are expressing functional response elements for PPARγ and RAR/RXR receptors (Kilgore et al, 1997). In this study we show that CIG increases iodide uptake in combination with tRA in MCF-7 cells, whereas CIG alone did not have any effect. CIG and tRA induced NIS expression seems to be specific to MCF-7 cells as we did not observe iodide uptake in HeLa cells (cervical carcinoma cell line) when these were stimulated with CIG and tRA upto 48 h.

Material and Methods

Cell Culture and Chemicals:

All the cell lines used in this study were obtained from German collection of microorganisms and cell cultures (DSMZ, Braunschweig, Germany, DSMZ Nr.: MCF-7 (ACC 115). MCF-7 cells were grown in RPMI 1640 medium, 10% FCS supplemented with 1 x non-essential amino acids, 0.01 mg/ml bovine insulin and 5% penicillin/streptomycin mixture. HeLa cell line was grown in in RPMI 1640 medium. All cell culture reagents unless otherwise stated were obtained from Invitrogen, Karlsruhe, Germany.

Ciglitazone (CIG) and *trans*-retinoic acid (tRA) were purchased from Biomol (Hambourg) and Sigma (Munich, Germany) respectively. For experimental purpose, CIG was dissolved in

DMSO as a 100 mM solution and tRA was dissolved in ethanol as a 33 mM stock. The stock solutions were stored at –20°C and further dilutions were prepared in growth medium just before the use.

Iodide Uptake Experiments:

Cells were grown in 24 well plates for 24 h. After 24 h the cells were washed and the compounds to be tested were added in fresh medium for the indicated period of time. For iodide uptake, the cells were at first washed twice with pre-warmed RPMI 1640 medium without FCS and then incubated in 1 ml RPMI 1640 without FCS, supplemented with 0.1 μCi/ml (specific activity: 10mCi/mmol) $Na^{125}I$ (Amersham Biosciences, Freiburg, Germany) and 10 μM NaI/well and cultured for 1h. To test for the specificity of NIS mediated iodine uptake, experiments were also performed in presence of 30 mM $KClO_4$ the strongest inhibitor of NIS. After incubation, cells were washed twice with ice cold RPMI 1640, lysed in 2% SDS (0.0625 M Tris-HCl, pH 6.8) SDS-PAGE, sample buffer containing 1U/μl benzonase (Merck KGaA, Darmstadt, Germany). ^{125}I uptake was measured in a gamma counter. An aliquot from each sample was used for determination of protein concentration by Bio-Rad DC Protein assay kit (Munich, Germany) to normalize for difference in cell number.

Statistical Analysis:

The statistical analysis was performed by paired T-test using Statview, version 5.0 (SAS Institute Inc. USA).

Short description of Figures

Fig. 1 shows the effect of CIG on tRA induced iodine uptake in breast cancer cells. Cells were treated with 1 μM tRA with or without 5 μM CIG for 48 h and iodide uptake assay was performed with (■) or without (□) 30 mM $NaClO_4$. Values are expressed as means ± SD (n = 3).

Fig. 2 shows that the effect of tRA and CIG on iodine uptake is cell selective. MCF-7 and HeLa cells were treated with 1 μM tRA with or without 5 μM CIG for 48 h. Iodine uptake assay was done in absence or presence of 30 mM $NaClO_4$ for 1 h. Values are expressed as means ± SD (n = 3).

Results:

Ciglitazone Enhances Iodine Uptake Induced by Retinoic Acids in MCF-7 Cells

MCF-7 cells were first treated with 1μM tRA at different time intervals. In pilot experiments, we determined that maximum uptake was achieved with 1μM tRA at 48 h (data not shown), consistent with a previous report (Kogai T et al, 2000). To examine whether the increase in iodine uptake is due to the effect of tRA alone or is a combined effect of tRA and CIG, MCF-7 cells were treated with 1 μM tRA in presence or absence of 5 μM CIG for 48 h. Iodide uptake assay showed that combined treatment of 5 μM CIG and 1 μM tRA increases iodine uptake 3.3 fold compared to 1 μM tRA alone. Treatment of cells with 1 μM tRA results in 1.5 fold increase in uptake compared to basal level. (Fig 1) . The iodine uptake was inhibited by 30 mM perchlorate, the specific inhibitor of NIS activity (Fig 1). These results indicated that

iodine uptake induced by tRA is enhanced by CIG and is mediated by the specific function of NIS because iodine uptake was blocked by the NIS inhibitor perchlorate. The viability of cells as observed by trypan blue staining were not effected by treatment of CIG and tRA (data not shown).

Effect of CIG and tRA on Iodine Uptake is Specific for MCF-7 cells

The induction of iodine uptake upon combined stimulation with CIG and tRA was also tested on HeLa in order to test the specific effect of these compounds on MCF-7. HeLa cells were treated with 1μM tRA with or without 5μM CIG for 48 h. Iodine uptake assay was performed in presence or absence of 30mM perchlorate. Unlike in MCF-7, we did not observe any induction of iodine uptake in HeLa cells after 48 h of treatment with tRA, CIG, tRA and CIG (Fig 2). The same result was obtained when HEK-293 was used in an analog experiment (data not shown). These results display the specific action of CIG in combination of tRA in MCF-7 cells in terms of iodine uptake.

Discussion:

We here report that CIG synergizes tRA induced iodine uptake in mammary carcinoma cell line MCF-7. MCF-7 cells treated together with CIG and tRA showed more than 3 fold increase in iodine uptake measured after 48 h in comparison to cells treated with tRA alone. MCF-7 cells showed about 20 fold increase in iodine uptake upon treatment with CIG and tRA in comparison to cells receiving no treatment. Treatment with tRA increases iodine uptake ~6 fold when compared to non treated cells.

trans-Retinoic acid (tRA) has been shown to induce iodine uptake in MCF-7 cells by increasing NIS expression whereby it is suggested that the action of RA is mediated by RAR/RXR receptors (Kogai T et al,. 2000). CIG mediated enhancement of iodine uptake activity and NIS mRNA level induced by tRA may be attributed to presence of functional response elements for PPARγ and RAR/RXR receptors in MCF-7 cells. We also observed that CIG induced increase in NIS expression which is mediated by tRA is specific in MCF-7 cells. There is no induction of iodine uptake in HeLa cells upon stimulation with tRA with or without CIG. tRA is previously also reported to induce no iodine uptake in prostate cancer cell line LNCaP, a choriocarcinoma cell line JEG-3 and two nonsmall cell lung cancer cell lines A549, H460 (Kogai T et al, 2000).

Up to date few other compounds are reported to induce functional NIS expression. NIS expression is known to be restored in a dedifferentiated thyroid carcinoma cell line by using the DNA demethylating agents like 5-azacytidine or sodium butyrate (Venkataraman GM et al, 1999). Histone deacetylase inhibitor, depsipeptide (FR90228) increases NIS expression in poorly differentiated thyroid carcinoma cell line.(Kitazono M et al, 2001).

The results presented here together with earlier reports indicate to use compounds such as tRA inducing functional NIS expression in organ specific manner together with thiazolidinediones such as ciglatizone in pharmaceutical preparations for diagnosis and treatment of of primary carcinomas and metastases of glandular carcinomas, in particular carcinomas of the salivary gland, the thyroid gland and of mammary carcinomas. Specifically, administration of radioiodine after systemic use of stimulatory compounds inducing NIS gene expression proves to be a valuable tool for diagnosis and treatment of the above mentioned tumors, for example some of the differentiated breast carcinoma. This approach could be studied for other responsive carcinomas of different origin.

References

Behr M, Schmitt TL, Espinoza CR, Loos U. Cloning of a functional promoter of the human sodium/iodide-symporter gene. Biochem J. 1998 Apr 15;331 (Pt 2):359-63.

Quandt, K. Frech, K. Karas, H. Wingender, E. and Werner, T. MatInd and MatInspector - New fast and versatile tools for detection of consensus matches in nucleotide sequence data. Nucleic Acids Research 23, 4878-4884 (1995)

Tazebay UH, Wapnir IL, Levy O, Dohan O, Zuckier LS, Zhao QH, Deng HF, Amenta PS, Fineberg S, Pestell RG, Carrasco N. The mammary gland iodide transporter is expressed during lactation and in breast cancer. Nat Med. 2000 Aug;6(8):871-8.

Mazzaferri EL, 1996 in The Thyroid, eds. Braverman LE and Utiger RD (Lippincott-Raven, Philadelphia), pp. 992-945.

Riedel C, Dohan O, De la Vieja A, Ginter CS, Carrasco N. Journey of the iodide transporter NIS: from its molecular identification to its clinical role in cancer. Trends Biochem Sci. 2001 Aug;26 (8):490-6.

De La Vieja A, Dohan O, Levy O, Carrasco N. Molecular analysis of the sodium/iodide symporter: impact on thyroid and extrathyroid pathophysiology. Physiol Rev. 2000 Jul;80(3):1083-105.

Heufelder AE, Morgenthaler N, Schipper ML, Joba W. Sodium iodide symporter-based strategies for diagnosis and treatment of thyroidal and nonthyroidal malignancies. Thyroid. 2001 Sep;11(9):839-47.

Nwankwo JO, Robbins ME. Peroxisome proliferator-activated receptor- gamma expression in human malignant and normal brain, breast and prostate-derived cells. Prostaglandins Leukot Essent Fatty Acids. 2001 Apr-May;64(4-5):241-5.

Inoue K, Kawahito Y, Tsubouchi Y, Yamada R, Kohno M, Hosokawa Y, Katoh D, Bishop-Bailey D, Hla T, Sano H. Expression of peroxisome proliferator-activated receptor (PPAR)-gamma in human lung cancer. Anticancer Res. 2001 Jul-Aug;21(4A):2471-6.

Sugiyama Y, Murase K, Ikeda H. Mechanisms of thiazolidinedione derivatives for hypoglycemic and insulin sensitizing effects. Nippon Rinsho. 2000 Feb;58(2):370-5. Review. Japanese.

Sato M, Yajima Y, Kawashima S, Tanaka K, Kagechika H. Synergistic potentiation of thiazolidinedione-induced ST 13 preadipocyte differentiation by RAR synergists. Biochem Biophys Res Commun. 2001 Jan 26;280(3):646-51.

Kitazono M, Robey R, Zhan Z, Sarlis NJ, Skarulis MC, Aikou T, Bates S, Fojo T. Low concentrations of the histone deacetylase inhibitor, depsipeptide (FR901228), increase expression of the Na(+)/I(-) symporter and iodine accumulation in poorlydifferentiated thyroid carcinoma cells. J Clin Endocrinol Metab. 2001 Jul;86(7):3430-5.

Titcomb MW, Gottardis MM, Pike JW, Allegretto EA. Sensitive and specific detection of retinoid receptor subtype proteins in cultured cell and tumor extracts. Mol Endocrinol. 1994 Jul;8(7):870-7.

Kilgore MW, Tate PL, Rai S, Sengoku E, Price TM. MCF-7 and T47D human breast cancer cells contain a functional peroxisomal response. Mol Cell Endocrinol. 1997 May 16;129(2):229-35.

Kogai T, Schultz JJ, Johnson LS, Huang M, Brent GA. Retinoic acid induces sodium/iodide symporter gene expression and radioiodide uptake in the MCF-7 breast cancer cell line. Proc Natl Acad Sci U S A. 2000 Jul 18;97(15):8519-24.

Venkataraman GM, Yatin M, Marcinek R, Ain KB. Restoration of iodide uptake in dedifferentiated thyroid carcinoma: relationship to human Na+/I-symporter gene methylation status. J Clin Endocrinol Metab. 1999 Jul;84(7):2449-57.

Schmitt TL, Espinoza CR, Loos U. Cloning and characterization of repressory and stimulatory DNA sequences upstream the Na/I-symporter gene promoter. Horm Metab Res. 2000 Jan;32(1):1-5.

Murphy GJ, Holder JC. PPAR-gamma agonists: therapeutic role in diabetes, inflammation and cancer. Trends Pharmacol Sci. 2000 Dec;21(12):469-74. Review.

Carrasco N. Iodide transport in the thyroid gland. Biochim Biophys Acta. 1993 Jun 8;1154(1):65-82.

Benson S, Padmanabhan S, Kurtz TW, Pershadsingh HA. Ligands for the peroxisome proliferator-activated receptor-gamma and the retinoid X receptor-alpha exert synergistic antiproliferative effects on human coronary artery smooth muscle cells. Mol Cell Biol Res Commun. 2000 Mar;3(3):159-64.

Patentansprüche

1. Zusammensetzung, enthaltend ein Thiazolidindion und einen Liganden für den RAR- oder RXR-Rezeptor, wobei dieser Ligand in der Lage ist, die Expression des NIS-Gens zu induzieren.

2. Zusammensetzung nach Anspruch 1, worin das Thiazolidindion ein Ligand für den PPARγ-Rezeptor ist.

3. Zusammensetzung nach Anspruch 1 oder 2, worin das Thiazolidindion ausgewählt ist aus der Gruppe bestehend aus Ciglitazon, Pioglitazon, Rosiglitazon oder Mischungen davon.

4. Zusammensetzung nach irgendeinem der vorhergehenden Ansprüche, worin der Ligand für den RAR- oder RXR-Rezeptor eine Retinsäure oder ein pharmakologisch verträgliches Derivat davon ist, insbesondere *trans*-Retinsäure oder ein Derivat davon.

5. Zusammensetzung nach irgendeinem der vorhergehenden Ansprüche, worin das pharmakologisch verträgliche Derivat ein Salz oder ein Ester ist, insbesondere ein Ester mit einer Alkansäure mit vorzugsweise 1 bis 4 C-Atomen.

6. Zusammensetzung nach irgendeinem der vorhergehenden Ansprüche, wobei die Zusammensetzung ferner einen Histondeacetylase-Inhibitor umfaßt.

7. Zusammensetzung nach Anspruch 6, worin der Histondeacetylase-Inhibitor Trichostatin A oder ein Butyrat, vorzugsweise Trichostatin A ist.

8. Zusammensetzung nach irgendeinem der Ansprüche 1 bis 7 als Diagnostikum, insbesondere als Diagnostikum für Primärtumore und Metastasen von glandulären, das NIS-Gen exprimierenden Karzinomen, insbesondere Speicheldrüsenkarzinomen, Schilddrüsenkarzinomen und Mammakarzinomen.

9. Zusammensetzung nach irgendeinem der Ansprüche 1 bis 8 als Medikament, insbesondere zur Behandlung von Primärtumoren und Metastasen von glandulären, das NIS-Gen exprimierenden Karzinomen, insbesondere Speicheldrüsenkarzinomen, Schilddrüsenkarzinomen und Mammakarzinomen.

10. Zusammensetzung nach Anspruch 9 oder 10 zur oralen oder parenteralen Verabreichung.

11. Zusammensetzung nach irgendeinem der Ansprüche 8 bis 10, wobei die Zusammensetzung ferner pharmakologisch unbedenkliche Träger- und/oder Hilfsstoffe umfaßt.

12. Kombinationspräparat in Form eines Kits, umfassend räumlich voneinander getrennt ein Thiazolidindion, einen Liganden für den RAR- oder RXR-Rezeptor, der Lage ist, die Expression des NIS-Gens zu induzieren, sowie radioaktives Iod oder Technetium, zur getrennten, gegebenenfalls zeitlich abgestuften Anwendung zur Diagnose und/oder Behandlung von Primärtumoren und Metastasen von glandulären, das NIS-Gen exprimierenden Karzinomen, insbesondere Speicheldrüsenkarzinomen, Schilddrüsenkarzinomen und Mammakarzinomen.

13. Präparat nach Anspruch 12, worin das Thiazolidindion ein Ligand für den PPARγ-Rezeptor ist.

14. Präparat nach Anspruch 12 oder 13, worin das Thiazolidindion ausgewählt ist aus der Gruppe bestehend aus Ciglitazon, Pioglitazon, Rosiglitazon oder Mischungen davon.

15. Präparat nach irgendeinem der Ansprüche 12 bis 14, worin der Ligand für den RAR- oder RXR-Rezeptor eine Retinsäure oder ein pharmakologisch verträgliches Derivat davon ist, insbesondere *trans*-Retinsäure oder ein Derivat davon.

16. Präparat nach irgendeinem der Ansprüche 12 bis 15, worin das pharmakologisch verträgliche Derivat ein Salz oder ein Ester ist, insbesondere ein Ester mit einer Alkansäure mit vorzugsweise 1 bis 4 C-Atomen.

17. Präparat nach irgendeinem der Ansprüche 12 bis 16, welches ferner einen Histondeacetylase-Inhibitor umfaßt.

18. Präparat nach Anspruch 17, worin der Histondeacetylase-Inhibitor Trichostatin A oder ein Butyrat, vorzugsweise Trichostatin A ist.

19. Präparat nach irgendeinem der Ansprüche 12 bis 18, worin das radioaktive Iod ^{123}I, ^{125}I und/oder ^{131}I ist, welches insbesondere in Form eines Alkali- oder Erdalkaliiodids, beispielsweise NaI vorliegt.

20. Verwendung eines Thiazolidindions zur Herstellung eines Medikaments zur Behandlung von Primärtumoren und Metastasen von glandulären, das NIS-Gen exprimierenden Karzinomen, insbesondere Speicheldrüsenkarzinomen, Schilddrüsenkarzinomen und Mammakarzinomen.

21. Verwendung eines Thiazolidindions zur Herstellung eines Diagnostikums, insbesondere eines Diagnostikums für Primärtumore und Metastasen von glandulären, das NIS-Gen exprimierenden Karzinomen, insbesondere

Speicheldrüsenkarzinomen, Schilddrüsenkarzinomen und Mammakarzinomen.

22. Verwendung nach Anspruch 20 oder 21, worin das Medikament zur oralen oder parenteralen Verabreichung ist.

23. Verfahren zur in vitro-Diagnose von Primärtumoren und Metastasen von glandulären, das NIS-Gen exprimierenden Karzinomen, insbesondere Speicheldrüsenkarzinomen, Schilddrüsenkarzinomen und Mammakarzinomen, welches die Schritte umfaßt:

a) Inkubieren von Zellen einer zu untersuchenden Probe mit einem Thiazolidindion und einem Liganden für den RAR- oder RXR-Rezeptor, der in der Lage ist, die Expression des NIS-Gens zu induzieren,
b) Inkubieren der unter a) erhaltenen Zellen mit einer Quelle für radioaktives Iod oder Technetium unter Bedingungen, die die Aufnahme des Iods oder Technetiums durch die Zellen erlauben, und
c) Bestimmen der Iod- oder Technetium-Aufnahme durch die Zellen.

24. Verfahren zur in vitro-Diagnose von Primärtumoren und Metastasen von glandulären, das NIS-Gen exprimierenden Karzinomen, insbesondere Speicheldrüsenkarzinomen, Schilddrüsenkarzinomen und Mammakarzinomen, welches die Schritte umfaßt:

a) Inkubieren von Zellen einer zu untersuchenden Probe mit einem Thiazolidindion und einem Liganden für den RAR- oder RXR-Rezeptor, der in der Lage ist, die Expression des NIS-Gens zu induzieren; und
b) Bestimmen der Expression von NIS-mRNA durch die Zellen.

25. Verfahren nach Anspruch 23, worin als radioaktives Iod ^{123}I, ^{125}I und/oder ^{131}I, eingesetzt wird, insbesondere in Form eines Alkaliiodids wie NaI.

26. Verfahren nach Anspruch 24, worin die Bestimmung der Expression von NIS-mRNA in Schritt b) mittels Reverse Transcriptase-Polymerasekettenreaktion (RT-PCR) erfolgt.

27. In-vitro-Diagnosesystem in Form eines Kits, umfassend ein Thiazolidindion und einen Liganden für den RAR- oder RXR-Rezeptor, wobei dieser Ligand in der Lage ist, die Expression des NIS-Gens zu induzieren, sowie gegebenenfalls einen Histondeacetylase-Inhibitor und eine Quelle für radioaktives Iod.

28. In vitro-Diagnosesystem nach Anspruch 27 zum Nachweis von Primärtumoren und Metastasen von glandulären, das NIS-Gen exprimierenden Karzinomen, insbesondere Speicheldrüsenkarzinomen, Schilddrüsenkarzinomen und Mammakarzinomen.

Fig. 1

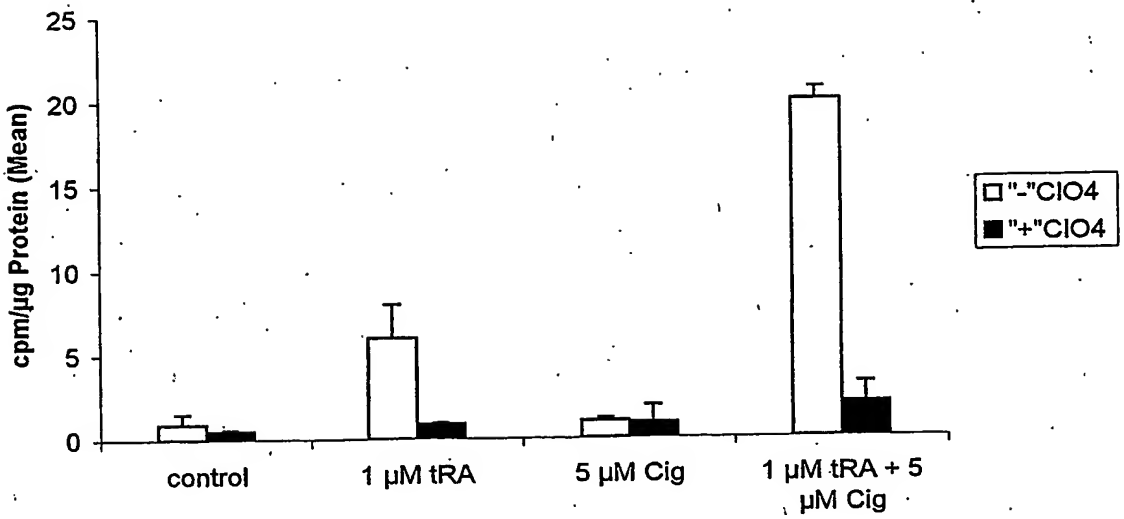
25
20
15
10
5
0
cpm/µg Protein (Mean)
control
1 µM tRA
5 µM Cig
1 µM tRA + 5 µM Cig
"-"ClO4
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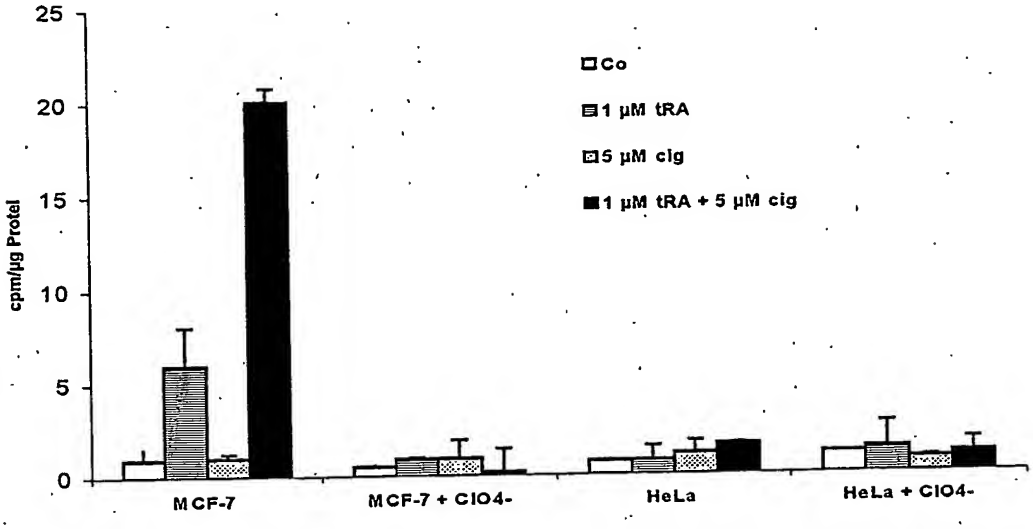
Fig. 2.


25
20
15
10
5
0
cpm/µg Protei
Co
1 µM tRA
5 µM cig
1 µM tRA + 5 µM cig
MCF-7
MCF-7 + ClO4-
HeLa
HeLa + ClO4-